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by Random Peptide Phage Display

PRINCIPAL INVESTIGATOR: Manuel Perucho, Ph.D.

CONTRACTING ORGANIZATION: The Burnham Institute
                          La Jolla, California 92037

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13. ABSTRACT *(Maximum 200 words)*

We proposed to develop a new approach for tumor-specific antibody detection based on Random Peptide Phage Display (RPPD) technology and verify whether such assays could be applied in the context of breast cancer, to determine the immunogenic spectrum of these patients. The relevance of our study was based on the obvious burden represented by this disease in terms of frequency and morbidity and the potential benefit that the development of new diagnostic, prognostic and therapeutic strategies can provide. During this granted period we have developed RPPD assays that effectively selects *mimotopes* specifically present in sera from cancer patients. Even more exciting is the fact that homology searches have revealed that most of the selected peptides are present in known cancer-related proteins, thus unveiling potential new candidate tumor-antigens. We have also observed cross-reactivity of certain peptides with other patients hosting the same tumor type. These results set an ideal scenario for 1) The application of this methodology in the individual analysis of breast cancer patients immune response and 2) the pursuit of large breast-cancer series screening in order to detect new tumor antigens commonly present in this patients, as anticipated in TASKS 2 and 3 of our proposal.

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A NEW APPROACH FOR THE IMMUNODIAGNOSTICS OF BREAST CANCER BY RANDOM PEPTIDE PHAGE DISPLAY

Manuel Perucho, PhD. Principal Investigator

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INTRODUCTION

In our initial proposal we intended to test a new approach for the development of diagnostic tools suitable for the early detection of breast cancer. Our approach was based on the assumption that the immune system detects at very early stages of the disease, the aberrant proteins associated with neoplastic transformation (1, 2, 3, 4). The immune system is responsible for targeting and elimination of alien elements including infectious agents and tumor cells developing a highly specific and complex response against its targets. The antibody spectrum present in the serum of a patient represents a pool of information that, once deciphered, becomes useful data in the development of cancer diagnostic, therapeutic, and prognostic solutions. We proposed to use peptide sequences compiled in RPPD libraries (5) as *mimotopes* for the identification of protein motifs recognized by antibodies present in the sera of breast cancer patients. We hypothesized that random peptide phage display (RPPD) libraries could be an optimal method for the isolation of cancer related epitopes from sera. This approach had proven successful in identifying ligands specific for autoimmune and infectious diseases (6, 7). RPPD libraries had been also proven useful for the characterization of proteins critical for the cell to cell and the cell to extracellular matrix interactions (8, 9, 10, 11) and for the development of selective organ targeting (12, 13). If succesful, the high sensitivity, specificity and speed of our approach should represent a remarkable breakthrough in the field of cancer immunodiagnostics. Our aim was to apply this strategy to test the hypothesis that tumor specific antigens of breast cancer patients can be detected by RPPD. After a first stage of collecting the necessary sera from breast cancer patients and working out the experimental conditions for such assays, as prerequisites for the isolation of epitopes recognized by sera antibodies of breast cancer, we planned to undertake an extensive study in order to determine if some of these tumor-specific antigens were shared by a significant number of cancer patients. Such achievement should justify the initiation of translational studies in order to evaluate the clinical significance of detecting such antibodies.

BODY

TASK 1.   *To assemble a small panel of sera from breast cancer patients and design a method for the detection of phage displaying peptides with high affinity for immobilized human antibodies.*

MILESTONE 1. The establishment of the experimental conditions for the enrichment of phage specifically binding to breast cancer patient sera. The demonstration of the success in the task will be done by measuring the input/output ratio after biopanning of phage on positive and negative sera.

Panel of sera

We have collected a series of 48 breast cancer, 28 colorectal cancer and 24 normal healthy individuals sera, procured by the Midwestern and Eastern divisions of the Cooperative Human Tissue Network (CHTN) of the NCI. All sera were drawn from patients at the time of surgery. Because the previous availability of sera from colorectal cancer (CRC) patients at the start of the project, the first experiments that we carried out to work the experimental conditions to achieve specific binding to the sera antibodies were done with the CRC sera. However, the principle of the method, once worked out, should be generalizable to breast cancer patients. During the period of these experiments, we have received and accumulated 48 sera from breast cancer patients to continue with the next steps of the project in the next year. We predict that at the middle of the second year we should have accumulated sufficient number of sera to allow the last step of the project (Task 3). We have used already the breast cancer sera as controls to show the specificity of phage binding to the cancer patient serum.

Isolation of phage displayed peptides selected by high affinity human antibodies.

In order to select peptides specific for antibodies from each patient serum, we developed a four-round procedure of phage panning. We used 7-mer, 8-mer, 9-mer cyclic and a 12-mer linear peptide libraries (14) of variable peptide length or a mixture of several libraries for the affinity selection of IgG and IgM immunoglobulins from cancer patients sera. Each round of the protocol consisted of an initial step of preadsorption of the phage libraries on immunoglobulins from healthy individuals in order to minimize further selection of non-cancer related antibodies, followed by affinity selection of the remaining antibodies present in the patient serum. Phage particles were eluted from the bound antibodies and subsequently amplified in bacteria. The amount of IgG or IgM immunoglobulins from healthy individuals used for preadsorption in each round of selection was fourfold higher than that from cancer patients. The binding results were quantified as the number of colony-forming units (cfu) produced by bacteria infected with the phage bound to normal and cancer patient sera antibodies. The panning procedure was considered successful when the number of cfu obtained after the fourth round of selection was at least twofold higher than those derived from healthy individuals control antibodies (Figure1, Appendix 1).

Characterization of selected peptides

The colonies derived from the patient antibodies were grown overnight and phage were purified by double precipitation with polyethylene-glycol. Phage DNAs were isolated and sequenced.

Assessment of the specificity of selected peptides.

In order to verify whether the isolated peptides were specific for CRC, we cloned the cDNAs encoding two of them, TGVRGQRISQ (patient A) and MKQSGHHRSE (patient B), in GST fusion vectors. We then purified the proteins

expressed in bacteria in order to screen a large number of sera by ELISA. Patient A peptide was present in 9 of the 18 phage particles isolated after selection on his own IgG. Likewise, patient B peptide was selected on IgG antibodies. This peptide had a KXGHH motif, which was also found in 9 of the 18 phage isolated in this case. Randomly selected peptides from unselected libraries were also expressed as GST fusions to be used as controls for non-specific binding. Only peptides isolated from phage libraries by affinity selection on cancer sera, when expressed as GST fusion proteins, bound IgG antibodies from their corresponding cancer sera in the ELISA (Figure 2 Appendix 1).

Next, we screened sera from carriers of different types of cancer and from healthy individuals to test whether they would bind to the GST-peptide fusion proteins. Nineteen of these patients had CRC, 48 had breast cancer, and 24 additional sera were obtained from healthy individuals. Three out of 19 sera from CRC patients bound to either GST-fusion protein derived from patient A or B peptides in the ELISA. Two CRC sera bound patient A peptide and one bound patient B peptide. ELISA results were considered positive whenever the binding signal to either peptide was at least twofold stronger than the signal obtained from the control peptides. None of the breast cancer or control sera reacted positively, thus showing that peptides TGVRGQRISQ (patient A) and MKQSGHHRSE (patient B) isolated from phage libraries are specific for CRC (p=0.049; *two tailed Fisher exact test*). These results provide solid evidence for the specificity of the epitopes represented by the RPPD selected peptides for the autologous cancer type.

Identification of tumor related protein sequences containing domains identical to RPPD selected peptides.

We performed a homology search with the peptide sequences isolated from patients A and B through library panning on IgG and IgM antibodies. For this purpose we used the publicly available protein database resources from the NCBI and the EMBL. We utilized the BLAST and FASTA algorithms. The first was accomplished using the Advanced BLAST module (15, 16) available on-line from the NCBI (URL: http://www.ncbi.nlm.nih.gov/BLAST/) which performs the search against the non-redundant database (GenBank Complementary DNA translations, PDB, SwissProt, PIR and PRF databases). For FASTA searches we chose the FASTA3 (17, 18) module from the EMBL outstation at EBI (Hinxton) also available on-line (URL:http://www2.ebi.ac.uk/fasta3/). This tool browses Swissprot, Trembl (translated EMBL database), and TremblNew (translated EMBL new database). The parameters of both modules were adjusted for optimal short-motif homology search. With BLAST we were able to limit the search to human sequences. In a typical homology search with the cancer specific peptides, some of the retrieved protein sequences corresponded to well known tumor-associated antigens recognized by cytotoxic T lymphocyte (CTL) response (Table 1, Appendix 2).

Among the 18 phage clones isolated on IgM antibodies of patient A, four contained the sequence QDLYSSA. An additional phage displayed a similar sequence, QDIFSSS (Table 1, Appendix 2). A BLAST search for the sequence QDLYSSA retrieved 5 different protein sequences. Among the highest scored sequences were those of the SART-1 tumor antigen and IgE autoantigen. These two protein sequences are 81% identical (GenBank acces # GI:2723284 and GI:2342526) and contain the motif QDLYS. Another sequence, FQSPK, represented by two of the isolated phage was identical to a fragment present in three components of the fucosyltransferases family, FUT3, FUT5 and FUT6 (19), as shown in the FASTA search. These three genes share more than 80% of sequence identity. They are involved in the synthesis of the Lewis histo-blood group of antigens. Two members of this group, sialyl Lewis x (sLex) and sialyl Lewis a (sLea), are well characterized tumor-antigens. Different carcinoma and leukemic cells display a substantial increase of sLex and/or sLea expression (20, 21, 22, 23).

A large body of evidence supports the role of these antigens in gastrointestinal cancer as early tumor markers and indicators of prognosis, in terms of tumor metastasis, recurrence and patient survival (24, 25). The relevance of FUT genes in the synthesis and expression of selectin ligands is corroborated by the abrogation of the metastatic behavior of CRC cells after stable transfection of antisense FUT3 sequences into these cells followed by a dramatic drop of the transcript (26, 27). In the same line of evidence, sLex antigen is reportedly overexpressed in breast tumor cells and this overexpression is positively correlated with the amount of FUT6 messenger RNA (28) (27). It is also noteworthy that an excessive overexpression of sialyl Lex elicits NK cell response against tumor cells rather than facilitate their hematogenous disemination (29)

Among the peptide sequences isolated on patient D, peptide QSLDHSSC matched with the highest score to a fragment of the MUC2 protein. Another well known cancer antigen, the carcinoembrionary antigen (CEA) (30), was among several equally scored sequences homologous to peptide YSWRAT. Peptide PQGWLGV showed homology with the melanoma antigen gp100 among other related sequences of this family. The peptide sequence NERSEAR matched P120 catenin. Surprisingly, the similarity search for two unrelated peptide sequences PGHVRGTLGR and YVDTLSKL recognized by patient D IgM antibodies, produced matches with two different domains within the same protein, neogenin (NGN), albeit not with exceptionally high scores.

The homology search for sequences binding to IgG antibodies from cancer patient sera yielded MUC4 (peptide QNPGETSKMN), a putative tumor suppressor gene, FAT, and two different cancer/testis antigen group members, MAGE C1 and CT7 (peptide TNVISYTPSSLY). We finally performed homology searches with each of 10 peptide sequences isolated from healthy individuals.

Two peptide sequences showed homology to portions of the variable regions of IgG and IgM immunoglobulins. This result could reflect the fact that these peptides represent idiotypic determinants present on normal immunoglobulins. The eight other peptide sequences yielded no biologically relevant hits.

The considerable amount of tumor related, and particularly, CRC associated antigenic proteins retrieved from our computational analysis, provides an unanticipated strong support for the biological relevance of the RPPD selection assay. Moreover, the presence within the retrieved tumor antigens of previously characterized tumor antigens such as CEA, MUC2, MAGE, etc, supports the idoneity of this methodology in the assesment of the specific tumor-induced immune response in a particular patient. In the same line of thought, it suggests the potential of this methodology for the identification of new tumor antigens in diverse tumor models.

Experimental validation of computational results.

In order to experimentally verify our computational findings, we elected to test whether the products of genes unveiled through homology search of sequences obtained from RPPD libraries screening are overexpressed by the corresponding tumors. Multiplex RT-PCR of RNA samples from patient A showed overexpression of FUT5 mRNA in tumor cells (Figure 3, Appendix 1). This result makes it unlikely for this particular protein to be a random result retrieved in the homology search.

Conversely, analysis of the expression of SART 1 antigen, yielded no significant increase in the amount of mRNA in the corresponding tumor. Secondary structure analysis of SART-1 protein sequence showed that the fragment QDLYS, which matches the sequence of our selected peptide, represents a coiled region that is likely to be located on the surface of the protein and to constitute an antigenic determinant. In order to overcome the potential lack of exposure of the antigenic sequence due to conformational alterations of SART-1 in the construct, we cloned in frame a 75 aminoacid fragment of the SART-1 protein in a GST vector, with the QDLYS motif located in its central portion. IgM antibodies from patient A serum bound the purified fusion protein as shown by ELISA, while serum from patients B, C, D and 4 normal individuals did not (Figure 4, Appendix 1). This finding strongly suggests that SART-1 protein triggered an IgM response in patient A, despite the lack of overexpression at the RNA level.

Tumor-associated antigens often induce a host immune response because they are overexpressed in tumor cells. HER-2/neu (31), CEA (30), and PSA (prostate-specific antigen) (32) illustrate this phenomenon. Similarly, we have evidence of the overexpression of FUT5 at transcript level in the autologous tumor. This finding furtherly supports the RPPD CRC-related epitope selection and minimizes the possibility of a random retrieval of this protein sequence from

the database. SART-1 showed no altered mRNA in our study. While this might represent a limitation of our method, in terms of validation of the homology search results, the binding of autologous IgM to a recombinant fragment of SART-1, as evidenced by ELISA, strongly suggests that in this particular case, the candidate tumor antigen indeed induced the immunity of the patient despite not being overexpressed at the time of serum collection. Interestingly, an independent report of an otherwise identical sequence categorized it as an IgE autoantigen. Autoantigens may induce the production of autoantibodies without being overexpressed or mutated (33).

The homology detected between two different peptides selected from the same patient with different regions of the same molecule, NGN, and the cross-reactivity observed in the specificity assessment of peptides, TGVRGQRISQ (patient A) and MKQSGHHRSE (patient B) with other CRC patient sera, provide additional support for the biological relevance of the RPPD selection.

These two peptides are among a subset of sequences that showed no homology with any relevant proteins compiled in databases. However, these peptide sequences were specific for CRC, as shown by ELISA. Antibodies raised against native proteins frequently recognize discontinous epitopes (33). Thus those peptides might well contain inserts that either mimic discontinuous antigenic determinants of known tumor-associated antigens or represent yet unidentified tumor-associated antigens. Most of the peptides associated with IgG antibodies from CRC patients in this study probably were sequences mimicking discontinuous epitopes. In contrast, most of the peptides corresponding to IgM antibodies from our CRC patients represented linear or continuous antigenic determinants present in proteins known to induce humoral and CTL immune responses.

The validity of our method is furtherly supported by the lack of homology with tumor associated antigens observed among 10 peptide sequences selected after one round of panning on normal serum. Remarkably, some of these sequences were similar to fragments of the variable regions of the heavy chains of IgG and IgM immunoglobulins. This suggests that the phage display approach can also detect anti-idiotypic antibodies present in normal human serum.

RPPD as a method for tumor antigen detection.

The data obtained upon accomplishment of TASK1 from our original proposal, conclusively shows that RPPDL and protein databases can be used for the identification of tumor associated antigens that induce humoral or cellular immune response in cancer patients. RPPDL assay provides the researcher with a list of peptide sequences or immunoprint, recognized by antibodies present in the serum of a patient. The time span required to complete the process is short (~ 1 week). This represents a considerable improvement compared to other existing methods like the serological analysis of recombinant cDNA expression libraries (SEREX). In this

approach the patient sera is used to screen prokaryotically expressed cDNA libraries prepared from autologous tumor specimens. This approach requires the construction of good quality cDNA expression libraries from the autologous tumors, what makes it inefficient for the screening of large series of sera samples. The RPPD method is faster, less laborious and in addition does not require the natural antigen to be present.

TASK 2.   *To screen a number of currently available random peptide phage display (RPPD) libraries and characterize peptide consensus sequences that show specific binding to antibodies present in breast cancer patients.*

MILESTONE 2. *The isolation of phage specifically binding to antibodies present in the sera of breast cancer patients but not in the sera of healthy individuals. The characterization of the sequence of common oligopeptides that bind to two initial breast cancer patient sera, but not to sera from healthy donors.*

As a preliminary requisite for the accomplishment of the main results contemplated in task 1, we have developed a suitable method based on RPPD library screening for the characterization of the specific immune response of colorectal cancer patients. The efficiency, specificity and speed of our method should enable us to run screening studies on different tumor models.

We have now gathered a collection of 48 sera withdrawn from breast carcinoma patients, part of which have been already used as controls in the development of the assay. Once the experimental conditions have been worked out for CRC, we intend to demonstrated the applicability of the approach for breast cancer. As shown for the specific antigens for CRC detected by the RPPDL approach, we expect to detect differences in the spectrum of immunogens in breast cancer. We are thus in a position to carry out succesfully the planned final stages of the project (Task 3).

TASK 3.   *To study the generality of the occurrence of these breast tumor specific antibodies by searching for their presence in a large panel of sera from breast cancer patients.*

MILESTONE 3. *The identification of phage displaying peptides specifically recognized by the antibodies from a majority of breast cancer patients.*

This section will be addressed in the next funding period of this proposal.

KEY RESEARCH ACCOMPLISHMENTS

- Development of a method for selection of peptides with high affinity for human antibodies by means of RPPD libraries.

- Characterization of selected peptides isolated from cancer patients sera.

- ELISA evidence for the specificity of the isolated peptides for cancer sera and for the autologous tumor type.

- Computational confirmation of the mimotope nature of isolated peptides.

- Identification of proteins, some of them already known tumor antigens, that contain domains homologous to the selected peptides.

REPORTABLE OUTCOMES

- A publication in preparation to be submitted to *Nature Biotechnology* reporting the assay as an efficient method for retrieval of tumor antigens from cancer patients.

- Application for a second term of financial support in order to fulfill the application of the method to breast cancer. Considering the so far attained results, we should be also able to select, isolate and characterize peptides specific for breast cancer. Some of these peptides are expected to be homologous to domains of proteins compiled in public databases. Among these, some should be already known breast-cancer related tumor-antigens. Others will unveil potentially new tumor antigens. Screening of larger series of sera should ensue in order to establish which of these antigens are present in a significant proportion of patients. Future projects should address the physiological relevance and potential application in diagnostic, therapeutic or preventive strategies of these immunogens.

CONCLUSIONS

We have now successfully accomplished the initial stage of our project. The attained results show that, as hypothesized in our original proposal, RPPD is a suitable method for the individual characterization of the specific immune response unleashed against autologous tumors. We have optimized a new assay that upon implementation in the context of breast cancer, should enable us to decode the tumor-related immune response in a particular breast cancer patient. Eventually we will undertake the screening of large series of patients in order to identify novel tumor antigens widely present in this group of patients. Although additional projects should verify the physiological relevance of those findings, both aims unequivocally share clinical interest.

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APPENDICES

Appendix 1
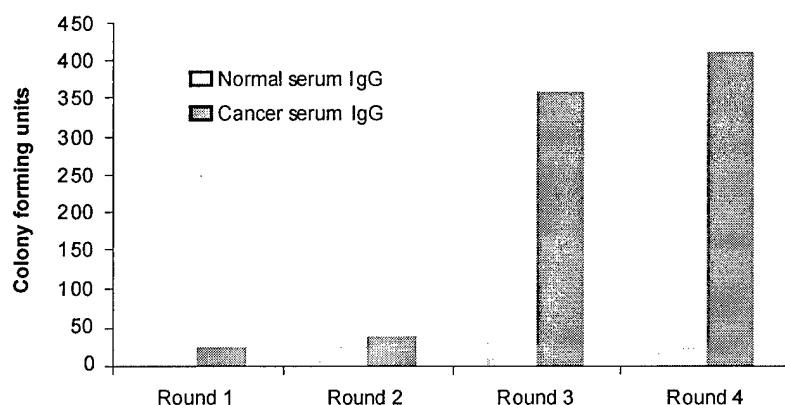
Figure 1. The graph shows the progressive enrichment of a RPPD library with phage particles that bind specifically to IgG antibodies from the serum of cancer patient A after four rounds of biopanning. After each round of preadsorption on excess of normal serum IgG and affinity selection on patient A IgG, amplified phage particles were purified and quantified. Equal amounts of phage (10^7 cfu) were incubated for 1 hour with normal serum IgG and cancer patient A IgG immobilized on protein G agarose. After washing out unbound phage, the remaining phage were eluted and used to infect bacteria. Aliquots of infected bacteria were spread on LB plates containing tetracycline. The next day, the number of colonies on plates corresponding to normal IgG and cancer patient A IgG were quantified.
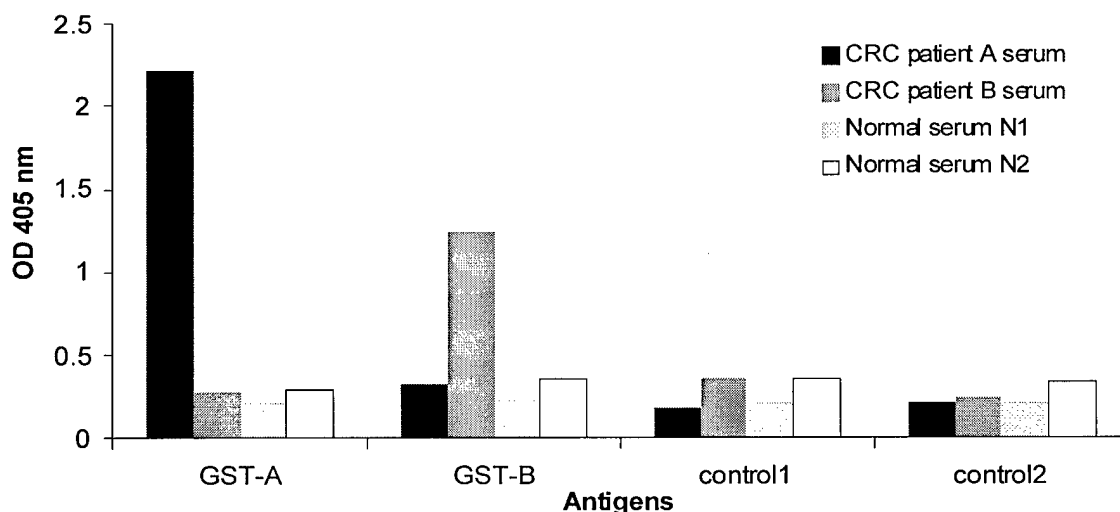
Figure 2. The graph depicts the specificity of binding of RPPD selected peptides to serum from autologous patients as evidenced by ELISA. Conversely, the sera from normal individuals display no affinity for these mimotopes. Purified GST-peptide fusion antigens were used to coat ELISA microplates. After blocking with 3% bovine serum albumin (BSA) in PBS the antigens were incubated overnight with cancer patient and normal individual sera diluted 1:200 with 3% BSA in PBS. The binding was detected using goat anti-human IgG antibodies conjugated with alkaline phosphatase and p-NPP as a substrate. GST-A and GST-B are TGVRGQRISQ and MKQSGHHRSE peptides expressed as GST fusion proteins. As control peptides we used arbitrarily chosen peptides isolated from phage libraries and expressed as GST fusion proteins. The average of three measurements is shown.
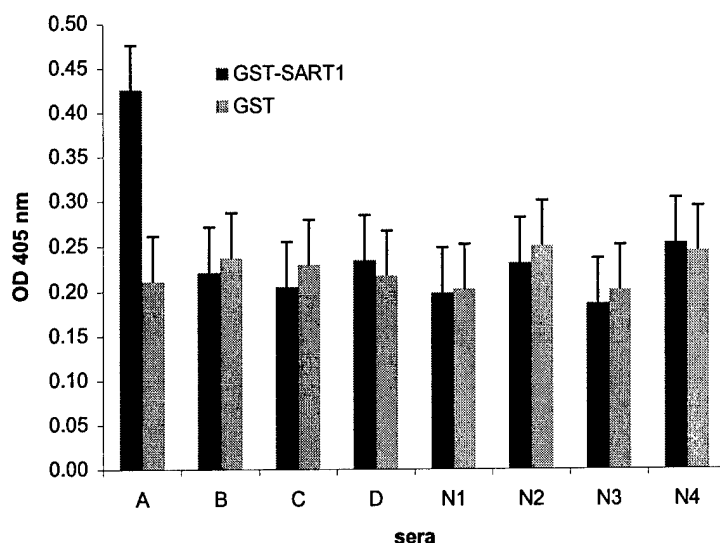
Figure 4. The graph illustrates the significant reactivity of patient A serum to a SART-1 antigen recombinant construct as shown in ELISA. Binding to the SART-1 GST fusion protein of serum IgM antibodies from cancer patients A, B, C and D and from four healthy individuals (N1-N4) is charted. Averages and the standard error of the mean of three measurements are shown.
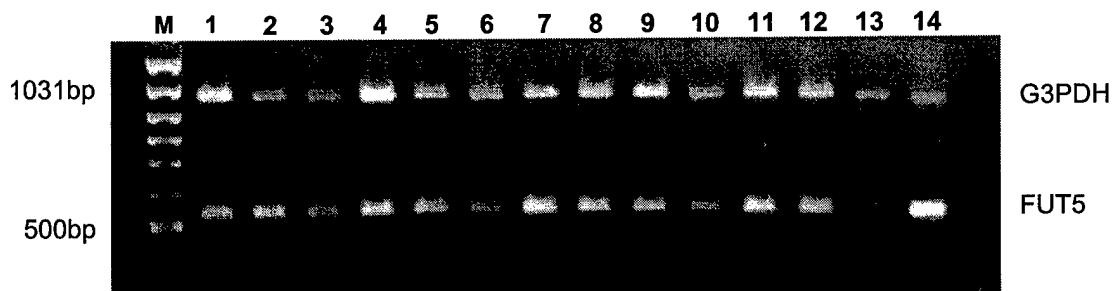
Figure 3. The dramatic difference in expression of FUT5 in patient A CRC tumor sample (lane 14) correlates with the selection of a mimotope from this patient that is contained in FUT5 protein sequence. This finding supports the biological relevance of our method. Simultaneous RT-PCR amplification of FUT5 and G3PDH gene fragments from normal colon tissue and colon tumor tissue mRNA. PCR products were electrophoresed on a 1% agarose gel. Numbers 1, 3, 5, 7, 9 and 11 are RT-PCR products from normal colon samples mRNA. Numbers 2, 4, 6, 8, 10 and 12 are RT-PCR products from corresponding colon tumor samples. Number 13 is the PCR product obtained from a cDNA of normal colon obtained from Clontech. Number 14 is the RT-PCR product obtained from the tumor of colon cancer patient A.

APPENDIX 2

Table1

Patient	Serum Ab.	Peptidic insert sequences from "*biopanning*"-selected phages			Homology search results	
					Acc. #	Sequence name
A	IgG	TGVRGQRISQ(9)	[a]QNPGETSKMN(6)	KYRWYK(3)	[a] 3551821	Mucin 4
A	IgM	AVHFPD	DLITPGD(2)	AEPPFEF(2)	[b] 2723284	SART-1
		PSKAAYVV (3)	[b]QD*L*YSSA(3)	QD*IF*SSS	[c] 1730136	FUT 6 *(Fucosyltransferase)*
		MSSVMTY	[c]FQSPK(2)	ASHQNRP	[c] 1730135	FUT5
		FRQAAS			[c] 121137	FUT3
B	IgG	FSRRAQQVGAK(3)	KAYGHHLSAE(2)	KSNKCF		
		DHNRSM	GLGVGHK	MKQSGHHRSE		
		SHNRVSN	SYSGYWHSWI	WTRRPYDELIV		
		KKGMGHHGNG(3)	FGAKSHGHHR			
C	IgG	KENGR**SPTHS**(10)	[d]**SPTHPQ**(5)	GRSNKSG(2)	[d] 422832	Mucin 6
		GRRNKSG				
D	IgG	VPWSKPWWTQTA	GHNNHNRHHPFS	S**NVR**SFDNPITM	[e] 4885475	MAGE C1
		ANT**PWSK**TLRP(2)	IPLPPPSRPFFK(2)	HNTRNWTLPPTY	[e] 3252909	cancer/testis antigen CT8
		SNVISYPDRVKM	GN**PWSK**QINIDR	VNTTSYNMRPNL(2)	[e] 4885229	FAT tumor suppresor
		L**PWSK**LSSPSRN	SNVKNYMAIPTR	QLHPHNLHSPWR		
		TLHTTHSPFKPI	NYEPVPRGARPH	TDAA**PWSK**VTTR(2)		
		GKSLHGSHHPWQ	**SNVIS**FRHASPT	[e]T**NVIS**YTPSSLY		
D	IgM	LVYPQQ	[f]QSLDHSSC(5)	LNPQSPRD(4)	[f] 186398	MUC2
		[g]YSWRAT(4)	[h]NERSEAR	HFHHLAVRGR	[g] 180241	CEA
		[i]PQGWLGV	GTVEDP	PTRWGARLVK	[h] 3152863	p120 catenin isoform 4ABC
		GGRWNR(2)	PETTDK(2)	RTGRMWR	[i] 547230	Melanoma antigen gp100
		RGQSLA	[j]PGHVRGTLGR(2)	AVRRPD(4)	[j] 1621607	Neogenin
		QRLAAGFH	QLAETLF	GRKTELF	[k] 1621607	Neogenin
		YLASPFE	FRVARAA	[k]YVDTLSKL	[l] 553465	Ig heavy chain
		VVGLVP(2)	QIQLSGG	[l]VKNRGR		
Normal	IgG	[m]DIRLSAQL	[n]SWSGYYTY	TNGVHHGR	[m] 542874	Ig kappa chain V region L13
		GLYNFMGK	RRTDYLLNGI	DPTVSESS	[n] 346202	Ig heavy chain V region
		APQGYLFK	NQHILSVG	[o]SIAAAVH	[o] 1914757	Immunoglobulin heavy chain
		RANKEPAT	ESSTKSE			

1) All other sequenced peptides than those selected from patient D IgG Ab., are flanked by cistein residues.
2) Numbers in parenthesis indicate the number of identical copies of the same phage after selection.
3) Highlighted in bold or underlined are conserved motifs present in different phages.
4) Highlighted in italics are conservative residue substitutions present in different phages.
5) Shadowed cells contain those peptide sequences yielding biologically relevant, cancer-related homologies.
6) Superscript letters relate peptide sequences with their relevant homologous sequences
7) The accession numbers provided correspond to the genbank (NCBI) entry reference of each sequence